

# CLAIMS

1. An enzyme that releases an amino acid having a glycated  $\alpha$ -amino group from a glycated protein or a glycated peptide.
2. An enzyme according to claim 1 derived from a bacterial strain of a genus *Corynebacterium*.
3. An enzyme according to claim 2, wherein the bacterial strain of the genus *Corynebacterium* is *Corynebacterium ureolyticum* KDK1002 (FERM P-17135).
4. An enzyme according to claim 1 derived from a bacterial strain of a genus *Pseudomonas*.
5. An enzyme according to claim 4, wherein the bacterial strain of the genus *Pseudomonas* is *Pseudomonas alcaligenes* KDK1001 (FERM P-17133).
6. An enzyme according to any one of claims 1 to 5, wherein the amino acid having a glycated  $\alpha$ -amino group to be released is valine having a glycated  $\alpha$ -amino group.
7. A method of determining a glycated protein or a glycated peptide comprising:
  - degrading a glycated protein or a glycated peptide with an enzyme to give a degradation product;
  - causing a redox reaction between the degradation product and a fructosyl amino acid oxidase; and
  - determining the redox reaction so as to determine the amount of the glycated protein or the glycated peptide,wherein an enzyme according to any one of claims 1 to 5 is used as the enzyme.
8. A method according to claim 7, wherein the glycated protein to be determined is glycated hemoglobin.

- Sub 92
- 5 9 A kit for determining a glycated protein or a glycated peptide comprising:  
 a protease;  
 a fructosyl amino acid oxidase;  
 a peroxidase; and  
 a substrate that is oxidized through a reaction with the peroxidase,  
 wherein the protease comprises an enzyme according to any one of  
 claims 1 to 5.

- 10 10 A bacterial strain of a genus *Corynebacterium* which produces an  
 enzyme which releases an amino acid having a glycated  $\alpha$ -amino group from a  
 glycated protein or a glycated peptide

- 11 A bacterial strain according to claim 10,  
 wherein the bacterial strain of the genus *Corynebacterium* is  
 15 *Corynebacterium ureolyticum* KDK1002 (FERM P-17135).

- 12 A bacterial strain of a genus *Pseudomonas* which produces an enzyme  
 which releases an amino acid having a glycated  $\alpha$ -amino group from a  
 glycated protein or a glycated peptide.  
 20 13 A bacterial strain according to claim 12,  
 wherein the bacterial strain of the genus *Pseudomonas* is  
*Pseudomonas alcaligenes* KDK1001 (FERM P-17133).

- Sub 93
- 25 14 A substrate for detecting an enzyme according to any one of claims 1 to 5  
 or for determining an activity of the enzyme comprising:  
 an amino acid; and  
 a detection group,  
 wherein the amino acid has a glycated  $\alpha$ -amino group,  
 the detection group is bound to an  $\alpha$ -carboxyl group of the amino acid  
 30 by an amide linkage or ester linkage, and  
 the detection group cannot be detected in its binding state whereas it  
 can be detected if released.

- 35 15 A substrate according to claim 14,  
 wherein the detection group is at least one selected from the group  
 consisting of paranitroanilide, paranitrophenol,  $\beta$ -naphthylamide, 4-methoxy-  
 $\beta$ -naphthylamide, and 4-methyl-coumaryl-7-amide.